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PRE-FORMULATION ASSESSMENT: FRUIT OF *CUMINUMCYMINUM*, LINN.

Shanthi.A[1], R. Radha[1]

[1]Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai,Tamilnadu

E-mail of corresponding author: shanthipharm@rediffmail.com

ABSTRACT

***Cuminumcyminum*, (Linn.)Correa;** belonging to the family**Umbelliferae**is commonly known as *Cumin* (Eng.) and Jira (Hindi). It is a glabrous, annual herb. Cultivated as a cold season crop on the plains and as summer crop on the hills in Northern India, a native of west Asia. It has the traditional value for curing various ailments like Asthma, fever, skin diseases, leprosy and also as a helminthiasis. This traditionally useful part (fruit) was standardized based on the pharmacognostic, physico-chemical and chromatographic conditions. Quality assessment by determining the limits of Microbials, heavy metals, pesticides and Aflatoxin as per WHO guidelines. The report states that the sample taken was standard under AyurvedicPharmacopoeial limits and WHO. The preliminary phytochemical screening confirms the presence of Essential oils, Glycosides, sterols and proteins. HPTLC finger print states the presence of nine different constituents and determination of total volatile oil content by Gas chromatography was found to be about 0.887%w/w. From this the report confirms that the sample was standard enough to use in the developing a herbal formulation.

Keywords: *Cuminumcyminum*, standardization, phytochemical screening, chromatographic identification and total volatile oil estimation by GC

INTRODUCTION

Herb is a plant that is valued for flavor, scent, medicinal or other qualities other than its food value[1]. Over the last few years, researchers have aimed at identifying and validating plant derived substances for the treatment of various diseases. Interestingly, it is estimated that more than 25% of modern medicines are directly or indirectly derived from plants[2]. With the emerging interest in the world to adopt and study the traditional system and to exploit their potentials based on different health care systems, the evaluation of the rich heritage of the traditional medicine is essential[3].The general standardization protocols to determine the percentage of active medicaments could not be followed for Ayurvedic herbal preparations. The procedures have to be modified in order to make the preparation safe. The approach has to be made from raw materials to finished products evaluation for the successful outcome. Which include the standardization of rawmaterial.

The drugs of plant origin especially of herbaceous nature are identified with their origin, common name, scientific

nomenature,family, geographical source, cultivation, collection, preservation, storage, macroscopy,microscopy, chemical composition, identity, purity, strength and assay, substitute andadulterants etc., The microscopic examination of root which includes Transverse sectionand Longitudinal section are made for identification[4]. *Cuminumcyminum* is commonlyknown as '*Cumin*' cultivated as a cold season crop on the plains and as summer crop on the hills in Northern India, a native of west Asia and Cultivated throughout India. Cumin is one of the constituents of siddha preparation *Attalicuranam*[5]. The ripe fruits have been used traditionally for curing various ailments like Asthma, fever, skin diseases, leprosy and also as a helminthiasis[6]. And it also used in neurological disorders[7], anti-depressant effects[8]. The present work was based on the standardization of *Cuminumcyminum* fruit as per WHO guidelines[9]. The complete standardization of this variety may be used for formulation development in future.

EXPERIMENTAL PART

1) Chemicals and reference drugs:

All the chemicals used in this present work were analytical grade in nature. The chromatographic estimations were done in the Asthagiri Herbal Research Foundation (Chennai) are chromatographic grade.

2) **Collection of plant materials**:

The roots of *Cuminumcyminum* were provided by M/S AnnaiAravindHerbals,Chennai. The sample was taken for authentication.

3) **Macroscopic evaluation**:

Organoleptic characters like colour, odour, taste, size, shape and other characters can be identified entirely or it fragment. This is authentication of crude drug with its genuine varierty[10].

4) **Microscopic evaluation**:

The microscopic appearance of the drug both in section view and in powdered form for its authentication with its genuine variety was performed[11].

5) Determination of foreign matter:

About 100g of the drug sample was spread out on a thin layer. The foreign matter was detected by inspection, separated and weighed. And the percentage of foreign matter was calculated[12].

6) **Determination of total ash**:

About 2g accurately weighed drug was incinerate in a tarred silica dish at a temperature not exceeding 450^0c until free form carbon, cooled and weighed. The percentage of ash obtained was calculated with reference to the air-dried drug[13].

7) Determination of acid-insoluble ash:

Total ash obtained boiled for 5 minute with 25ml of dilute hydrochloric acid; insoluble matter was collected in a Gooch crucible, or an ash less filter paper, again washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash was calculated with reference to the air dried drug.

8) Determination of water soluble ash:

The ash obtained was boiled for 5 minutes with 25ml of water; insoluble matter was collected in a Gooch crucible or on an ash less filter paper, wash with hot water, and ignited for 15minutes at a temperature not exceeding 450^0c. The weight of the insoluble matter obtained was subtracted from the weight of the ash; the different in the weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

9) Determination of alcohol soluble extractive:

About 5g of the air-dried drug was coarsely powdered and macerated with 100ml of Alcohol (specified strength) in a closed flask for 24hours, shake frequently during 6hours and allowed to stand for 18hours. Filtered rapidly, taking precautions against loss of solvent and evaporated 25ml of the filtrate to dryness in a

tarred flat bottomed shallow dish, and dried at 105^0C to constant weight and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air-dried drug.

10) Determination of water soluble extractive:

About 5g of the air-dried drug was coarsely powdered and macerated with 100ml of Chloroform water in a closed flask for 24hours, shake frequently during 6hours and allowed to stand for 18hours. Filtered rapidly, taking precautions against loss of solvent and evaporated 25ml of the filtrate to dryness in a tarred flat bottomed shallow dish, and dried at 105^0C to constant weight and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air-dried drug.

11) Determination of moisture content (loss on drying)

About 10 g of the drug accurately weighed and place in a tarred evaporating dish and dried at 105^0c for 5 hours, and weighed. The drying and continued and weighed at 1hour interval until difference between two successive weighing corresponds to not more than 0.25 percentage is achieved.

12) Quality parameters:

Limit test for microbial limits:

Microbial limits were detected for Total aerobic count, Total bacterial count, Total *Yeast* and *moulds*, Test for *Escherichia Coli, Salmonella species, Psueudomonasaerugenosa, Staphyllococusaureus* and Aflatoxins[14].

13) Pesticide residue:

A pesticide is any substance or mixture of substance intended for preventing, destroying or controlling any pest. Chromatographic estimation can be used for detecting and quantifying the pesticide limits in the crude drug was detected[15].

14) Determination of heavy metals:

The test for heavy metals is designed to determine the content of metallic impurities that are coloured by Sulphide ion, under specified conditions. The limit for heavy metals is indicated in the individual monograph in terms of parts of lead per million of substance (by weight). It includes the limit test for Arsenic, Lead, Mercury, and Cadmium[16].

15) Preliminary phytochemical screening:

Preliminary phytochemical screening for alkaloids, glycosides, tannins, terpenoids, phenols,

sterols, proteins, amino acids, volatile oils, flavonoids, saponins, fixed oils, essential oils, coumarins, sugars and others[17].

16) **Study of colour change under UV light:**

The powdered drug was studied for any colour change under UV light. The sample was divided into three parts and each was studied under ordinary light, short UV (254nm) and long UV (356nm). First part is observed as such, second part observed after treating with 50% Hydrochloride acid, and third part observed after treating with 50% Sodium hydroxide[18].

17) Chemical identity: TLC analysis of plant drugs[19].

About 4g of the sample was soaked in chloroform for 18 hours, boiled, filtered and concentrated to 10ml in standard flak**.**

20µl of the sample was applied on the Merck aluminium plate pre-coated with silicagel 60 F254 of 0.2mm thickness and the plate was developed in water: Aceto nitrile: methanol: ethyl acetate: hexane (1.5:5.0:0.5:1.5:1.5). The plate was dried and photographed under UV 254 and 366nm.

HPTLC fingerprint:

A Cammag HPTLC system equipped with a sample applicator Linomat IV, twin trough plate development chamber, TLC SCANNER II and Wincats used as an integration software 4.02 (Switzerland). TLC aluminium plates pre-coated with silica gel 60 F 254 (10x10cm, 0.2mm thick) were used. The estimation has been done using the following chromatographic conditions. The plate was dried and scanned at 200 -450nm

using Deuterium lamp the finger prints was developed.

18) Quantitative estimation:

Volatile oil determination was carried out by Gas chromatography. It is an excellent tool for the separation, characterization and quantitative estimation of volatile component of essential oil containing drugs. The identity of the components was assigned by comparing their GC retention times with those of authentic samples, as well as of the components of other essential oils.

GC was performed on a Varian gas chromatograph, model cx-3400, under the following conditions: carrier gas, hydrogen; injector and detector temperatures, 2208C and 2258C, respectively; using a capillary column (Supelcowax-10, 30 m _ 0.3 mm), with oven temperature programmed from 808C at 58C/min to 1508C, then at 78C/min to 2158C[20].

RESULTS

1. Authentication of the plant material:

Plant part used was authenticated by Prof. P. Jayaraman, National institute of herbal sciences, West Tambaram, Chennai. The voucher no: **PARC/2010/660**.

2. Determination of foreign matter:

Foreign matters were present in less than one percentage (Limit NMT 2%) the value was obtained from the triplicate of the analysis.

3. Macroscopic evaluation:

Type: Cremocarp.

Colour: brown with light coloured ridges.

Odour: Umbelliferous characteristic.

Taste: Spicy.

Size: 4-6 m long, 2 mm wide.

Shape: Ellipsoidal, elongated, tapering at both ends.

Extra features: Lateral: Slightly compressed.

Ridges: 5 primary and 4 secondary ridges.

The macroscopic image was given in **Fig 1: *Cuminumcyminum* fruit.**

4. Microscopic evaluation:

a. Transverse section:

It shows **Outer epidermis:** polygonal cells

Testa: brown coloured polygonal cells, bundles of lignified sclerenchymatous fibres

Inner epidermis: polygonal cell – regularly arranged.

Mesocarp: few layer of parenchyma with five vascular bundles – primary ridges and 6 vittaes under secondary ridges (4dorsal side, 2 on commissural surface).

Endocarp: polygonal cells with fixed oils, aleurone grains and rosette of calcium oxalate crystals, fibres in carpophore.

Fig 2: Transverse section of *Cuminumcyminum* fruit.

b. Powder:

Colour – Brown

Endosperm cells –Aleurone grains, fixed oil, calcium oxalate crystals.

Fragments of testa: brown polygonal cells.

Mesocarp: Fibrovascular elements, thick walled sclerenchymatous cells and large oil ducts.

Vessels – annular spiral thickening

Fig 3: Microscopy of*Cuminumcyminum* fruit.

5. Physico-chemical constants

The table No: 1 shows that the mean valueof physical constants such as LOD, Ash values and extractive values.

The report shows the presence of above values within the limits prescribed under Ayurvedic Pharmacopoeia of India, and the sample contain high proportion of water and alcohol soluble constituents.

6. Microbial determination:

The table No: 2 shows the presence of microbial limits of the sample.

And the report shows that as per the WHO standards, the plant material is free from microbial load and ranges within the standard limit and the Aflatoxin. And it was found to be safe for further use in formulation development.

7. Determination of pesticide

Pesticide analysis of the raw material states that the DDT, Benzene hexa chloride, Aldrin, Dialdrin, Lindane, Chloropurophos and Enoculphan type of pesticides are not detected in the drug sample.

ND – not detected (concentration less than the minimum detection limit even in ng/l units). From the above results it can be concluded that the plant material is totally safe and there is no traceable limit of pesticide in them.

8. Quality parameters:

The table No: 3 shows the presence of heavy metal limits of the sample.

The report shows that the raw material is free from Arsenic, Cadmium and Mercury and Lead is present within the standard limits.

9. Preliminary phytochemical screening:

Preliminary phytochemical screening indicates the presence of Essential oils, Glycosides, sterols and proteins.

10. Study of colour change under UV light:

The table No: 4 represents the colour change of the sample under normal, long and short UV.

Colour change was observed under ordinary light with acid and alkali medium with respect to powder alone and in short UV only in alkaline medium the colour change was observed and there was no change in colour was observed under long UVregion.

11. Chemical identity:

The table No. 5 indicates the Rf values obtained for the sample and the number of constituents identified with that respective Rf values.

The figure 3 gives the TLC image and the figure 4 gives the HPTLC finger print data. The result obtained from the HPTLC studies showed that, therewere nine spots were observed.

12.Quantification:

The total volatile oil content of the *Cumimuncyminum*fruit estimated by Gas Chromatography method was found to be about 0.887% w/w.

DISCUSSION

The macroscopic and the microscopic evaluation of drug establishing its quality control profile and according to WHO, botanical standardswere proposed as a protocol for the diagnosis.

The physic-chemical constants were lies within the AyurvedicPharmacopoeial limits states that the sample is free from adulterants and the quality evaluations for microbial, heavy metals states that they were present with in the WHO limits and the preliminary phytochemical evaluation states the presence of secondary metabolites and the HPTLC finger print reveals the number of constituents present and the quantification report states the purity of the sample based on the quantity of phytoconstituents.

CONCLUSION

The reports states that the *Cumimuncyminum*was authenticated based on its pharmacognostic, physico-chemical and phytochemical aspect. And the qualitative estimation impliessafety of the sample due to the absence of heavy metals, pesticides and microbes. Quantitative estimation states the therapeutically useful constituent (volatile oil) is present in it.Thus the *Cumimuncyminum* is valid for its therapeutic potency.And thus it can be used in the herbal formulation development.

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Table No: 1 Physical-chemical constants of *Cuminum cyminum* sample

Loss on Drying	Ash values			Extractive values	
	Total ash (w/w)	Acid insoluble ash (w/w)	Water soluble ash (w/w)	Alcohol Soluble (%w/w)	Water Soluble Extractive (%w/w)
4.49±0.081%	7.49±0.03%	1.023±0.045%	6.84±0.02%	37.1±0.04	50.06±0.22

Note: The tests are performed in triplicate and results given as Mean ± Standard Error Mean.

Table No: 2 Microbial determinations of *Cuminum cyminum*fruit.

Organism	Total bacterial count	Total plate count	Total yeast and moulds	Salmonella and E.coli	Aflatoxin	pesticides
Report	10 cfu/g	5 cfu/g	Nil	Negative	Nil	Not found

Note: cfu – colony forming units.

Table No: 3 reports of presence of heavy metals

S. No	Metallic element	Report observed (PPM)	Standard limit (PPM)
01	**Arsenic**	Nil	NMT 20 PPM
02	**Cadmium**	Nil	NMT 20 PPM
03	**Lead**	Less than 5 PPM	NMT 20 PPM
04	**Mercury**	Nil	NMT 20 PPM

Note: Tests are performed; ppm – parts per million

Table 4: Study of colour change under UV light

S. No	Sample	Ordinary Light	Short UV (254 nm)	Long UV (365 nm)
01	*Cuminum* Powder	Blackish brown	Green	Dark brown
02	Powder + 50% acid	Yellow	Green	Dark brown
03	Powder + 50% alkali	Brown	Pale green	Dark brown

Table 5: R_f values of *Cuminum cyminum* fruit.

S. No	No. of spots	R_f values	No. of constituents	λ_{max} value
01	**09**	0.03,0.05,0.20,0.29,0.39,0.41,0.67 ,0.71 and 0.86	1,2,6,8,11,12, 16,18and 21	282,285,352,348,335,329,325,332 and 268

Figure 1: Macroscopic image of Cuminum cyminum fruit

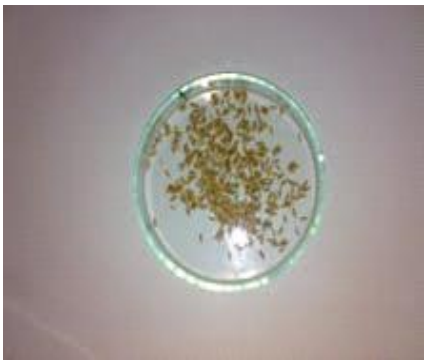
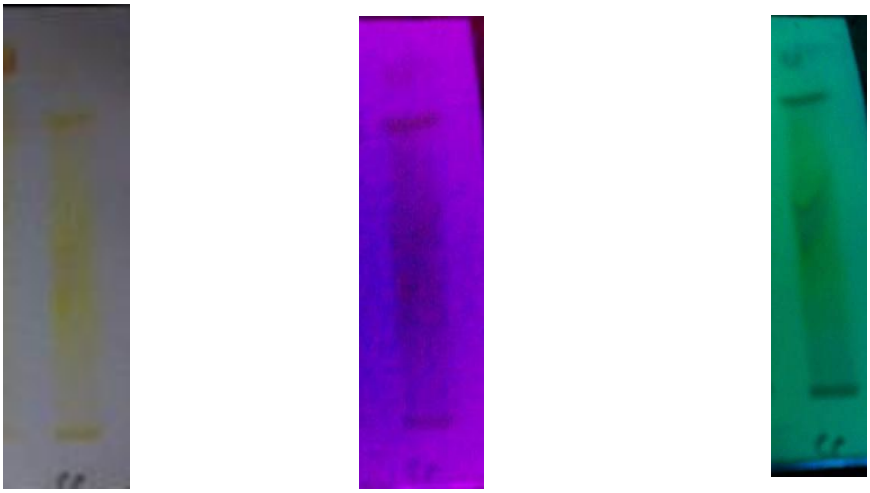


Figure 2: TLC images of Cuminumcyminum fruit.



Under normal light short UV (254nm) Long UV (365nm)

Figure 3: HPTLC finger print data of *Cuminum cyminum*fruit.